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CHANGES TAKING PLACE DURING THE SPOILAGE OF TOMATOES, WITH METHODS FOR DETECTING SPOILAGE IN TOMATO PRODUCTS.

By Raymond F. Bacon and P. B. Dunbar,
Assistant Chemists, Division of Foods.

INTRODUCTION.

Tomatoes contain, on the average, about 6.4 per cent total solids, of which 3.5 per cent is invert sugar, 0.5 per cent citric acid, 0.6 per cent ash, 0.9 per cent protein (N×6.25), 0.85 per cent crude fiber, and about 0.05 per cent fat. When tomatoes spoil as a result of the life processes of bacteria, yeasts, and molds the sugars are rapidly used up, being changed principally into acetic acid, lactic acid, alcohol, and carbon dioxid, the amounts of these substances depending on the types of organisms which are most active in the particular sample in question. During most classes of spoilage the citric acid is also rapidly decomposed, so that its amount serves as a valuable index in detecting decomposition. It is very easy to detect spoilage in tomato pulp or canned tomatoes, as such products, when perfectly sound, contain no volatile acids and a considerable percentage of citric acid and invert sugar, and when spoiled quite large amounts of volatile acids are present with little or no invert sugar or citric acid. But in such complex products as tomato ketchups and the various tomato sauces, of which sugar and acetic acid may be legitimate ingredients, and whose composition may vary within very wide limits according to the caprice of each manufacturer, it is evident that the ordinary analytical determinations give very little information as to the soundness of the tomatoes from which these products have been prepared. It has been found, however, that the condition of the tomatoes which enter into ketchups or other tomato products may be ascertained by determining the nature and quantity of the acids present and to a certain extent by the changes in the nitrogenous constituents. Also certain analytical ratios seem to give some information on this point, as will be developed more in detail in connection with the experimental data. A good ketchup or other tomato product is characterized by high citric acid content and no, or very

little, lactic acid, and a product prepared from spoiled tomatoes will probably have a low citric acid content (often none) and a high content of lactic acid.

It will probably be well to say a few words as to manufacturing methods in the tomato-products industry to show just how these spoiled tomatoes get into ketchup and how spoilage may be avoided. In preparing canned tomatoes there are many tomatoes which, because of their small size or other defects, are not suitable for canning purposes. These tomatoes, together with the skins, cores, and pieces of tomatoes from the canning factory, are cooked to prepare a tomato pulp. Many small canneries prepare pulp which is sold to ketchup manufacturers and in many factories pulp is prepared during the busy canning season to be worked up into ketchup during the dull seasons. In both instances it is quite common to store this pulp in barrels for considerable time. It has been conclusively demonstrated that it is, in practical work, almost impossible to get barrels tight enough to prevent contamination with organisms and consequent fermentation during such storage. If pulp must be stored, it should be sterilized in sealed lacquered tin cans (lacquered to prevent excessive solution of tin by the tomatoes, which are very active in this respect, probably because of their citric acid content). We are informed by some manufacturers, who have tried 5-gallon lacquered cans for this purpose, that the cost is really less than that of storage in barrels. With proper sorting to exclude decayed tomatoes from the factory, efficient washing, a prompt working up into pulp, and proper sterilization and storage in lacquered tin cans, there is little danger of decomposition in preparing tomato products.

There are two other processes of manufacture which should be mentioned. In one, the gravity process, the pulped tomatoes stand until the tomatoes rise (due largely to fermentation changes), and the clear liquid is drawn off from below. In another one, used principally for pastes, the tomatoes are mixed with a large amount of salt and allowed to ferment until the sugars, citric acid, and other easily changed substances have entirely disappeared, and this product is then put into cans without any sterilization. As both of these processes are based primarily on fermentative actions, tomato products prepared in these ways are necessarily composed of a decomposed vegetable product and, hence, such methods of manufacture appear to be indefensible.

EXAMINATION OF TOMATO PRODUCTS.

The acid of tomatoes has been called by various authors malic, citric, tartaric, and oxalic. The acid is actually citric acid, as shown by the following:

(1) Ordinary qualitative tests for oxalic acid on some tomato juice gave negative results; tomato juice to which 0.01 per cent of oxalic

acid was added gave a strong precipitate with calcium acetate. There is, therefore, less than 0.01 per cent of oxalic acid in tomatoes.

(2) Tomato juice tested for malic acid by the optical method with uranyl acetate (Circular 76, Bureau of Chemistry) gave a negative result. As this method is easily capable of detecting 0.02 per cent of malic acid, there must be less than this quantity present in tomato juice.

(3) The acids in the juice of fresh tomatoes were precipitated with a lead acetate solution, the precipitate was well washed with hot water, decomposed with hydrogen sulphid, the lead sulphid removed, and the barium salts of the acids prepared; 0.6870 gram of the barium salt, dried at 110° C., gave 0.6100 gram of barium sulphate, barium found, 52.3 per cent; calculated for $Ba_3(C_6H_5O_7)_2$, barium equals 52.15 per cent.

(4) The acids extracted from a tomato juice acidified with sulphuric acid by long extraction with ether gave in a 0.01 per cent solution in water a very strong precipitate with the Denigès reagent. The acids so extracted when in 10 per cent aqueous solution give no tests for malic or tartaric acids.

(5) Sound tomatoes contain no volatile acids. This question was tested on a very large number of samples of tomatoes of different origin, always with negative results.

Some idea of the general cause of tomato spoilage may be obtained by the following experiments:

Determinations on fresh and spoiled tomatoes.

Determination.	Fresh tomatoes.	Same tomatoes after spoilage.
I. Stood 2 weeks at room temperature:		
Polarization of filtered juice....°V..	−4.0	0.0
Total acids as citric....per cent..	.56	.06
Citric acid....do....	.58	None.
Ammonia by magnesium oxid method....do....	.038	.068
Volatile acids as acetic....do....	0.0	.462
Lactic acid....do....	0.0	.37
II. Stood 10 days in a rather warm place:		
Total solids....do....	5.8	3.66
Total nitrogen....do....	.15	.19
Total acids as citric....do....	.60	.59
Polariscopic reading of filtered juice....°V..	−6.15	0.0
Reducing sugars as invert....per cent..	3.3	0.0
Ammonia by magnesium oxid method....do....	.043	.056
Lactic acid....do....	0.0	.48
Volatile acids as acetic....do....	0.0	.120

The volatile acids were converted into the barium salt; barium, 43.6 per cent; calculated for barium butyrate, $Ba(C_4H_7O_2)_2$, 44 per cent. The volatile acids in the first case contained large amounts of butyric acid, and in the second case they consisted almost entirely of butyric acid, as was evident from the odor and from the odor of the ethyl ester which was prepared from these volatile acids. It appears that butyric acid is a rather common decomposition product when the

tomatoes spoil at a rather high temperature. At lower temperatures the acetic, lactic, and alcoholic fermentations seem to predominate.

A number of experiments have shown that the sugar of tomatoes is usually invert sugar with at times a slight excess of levulose. There does not seem to be enough regularity in this excess of levulose to enable one to use the determination of the kind of sugars as an index to the nature of the tomatoes which entered a tomato product. During the fermentation of tomato pulp both sugars are consumed.

Examination of commercial pulp and trimming stock.

Laboratory No.	Total solids.	Soluble solids.	Total acids as citric.	Volatile acids as acetic.	Lactic acid.	Citric acid.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
FS-32	7.34	5.83	0.8	[1] 0.2	0.57	0.0
FS-34	5.83	5.04	.6	.15	.2	.42
FS-36	4.06	.....	1.0	.22	.75	0.0
FS-78	.....	.....	.....	.....	.44	0.0
FS-73	.....	.....	.....	.....	.07	.52
FS-70	.....	.....	.....	.....	.42	.2
FS-69	.....	.....	.....	.....	.19	.28

[1] Contains butyric acid.

Description of sample and remarks.

FS-32. Commercial barrel pulp stored from season of 1909 to June, 1910. Yeasts and spores, 45 per 1/60 mm. Bacteria, 144,000,000 per cc. Molds in 40 per cent of fields. Skin and core pulp.

FS-34. Commercial barrel pulp stored about one year. But little decomposition. Yeasts and spores, 12 per 1/60 mm. Bacteria, 32,000,000 per cc. Molds in 40 per cent of the fields.

FS-36. Commercial barrel pulp one year old. All sugars fermented out. Odor very sharp and sour. Yeasts and spores, 130 per 1/60 mm. Bacteria, 228,000,000 per cc. Molds in 40 per cent of fields.

FS-78. Commercial trimmings affected by dry rot.

FS-73. Commercial pulp from trimmings. Good material, worked up at once. Yeasts and spores, 13 per 1/60 mm. Bacteria, 9,000,000 per cc. Molds in 20 per cent of fields.

FS-70. Trimming stock pulp which stood about 6 hours before being worked up. Yeasts and spores, 32 per 1/60 mm. Bacteria, 48,000,000 per cc. Molds in 45 per cent of fields.

FS-69. Sample of pulp from same stock as FS-70, but made up immediately. Yeasts and spores, 26 per 1/60 mm. Bacteria, 38,000,000 per cc. Molds in 29 per cent of fields.

(Microchemical examinations were made by B. J. Howard, Chief, Microchemical Laboratory.)

Examination of tomato pulp after inoculation.

Labratory No.	Total acids as citric.	Volatile acids as citric.	Ammonia nitrogen.		Lactic acid.	Citric acid.
			By magnesium oxid method.	Partition.		
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
FS-121	0.10	Distillate alkaline...	0.065	1 0.057 2 .010	0.27	0.02
FS-122	Alkaline.	.....do.....	.....	.....	.42	.02
FS-123	.11	.....do.....	.058	1 .062 2 .034	.08	.20
FS-124	.25	Distillate neutral....	.043	1 .054 2 .007	.11	.23

Description of sample and remarks.

FS-121. Whole tomato pulp inoculated with *Penicillium* and allowed to stand at room temperature for over two months.

FS-122. Whole tomato pulp inoculated with *B. coli.* Odor exceedingly foul after two months at room temperature.

FS-123. Whole tomato pulp inoculated with a mold and left standing at room temperature for two months.

FS-124. Whole tomato pulp inoculated with *Penicillium* sp. and left standing at room temperature for two months.

In the case of samples FS–121, 123, and 124 it is to be noted that the molds do not appear to form lactic acid in tomatoes as the bacteria do, but they do seem to remove the citric acid rapidly and to act on the nitrogenous constituents of the fruit in such a manner as to form a considerable quantity of free ammonia. The distillate from FS–121 was alkaline. However, when to 100 cc of filtered juice there were added 10 cc of 10 per cent sulphuric acid and this solution was distilled, the distillate was acid and required 0.9 cc of tenth-normal sodium hydroxid to neutralize it to phenolphthalein. The sample FS–122, similarly treated, gave a distillate which required 4.1 cc of tenth-normal sodium hydroxid, and the distillate from sample FS–123 required 22.4 cc, while that from FS–124 required 0.8 cc.

In some types of spoilage it is evident that there is present a certain quantity of combined volatile acids. A large number of good and of poor ketchups were tested for combined volatile acids. None of the known good ketchups contained combined volatile acids, while some of the poorer ones did. However, as some ketchups known to be prepared from decomposed materials did not show combined volatile acids, this determination can not be considered to be a certain index of decomposition, so its use was abandoned. For nitrogen partition, 100 cc of filtered juice was precipitated with 10 cc of 20 per cent lead acetate, and the total nitrogen determined in the precipitate (2) and the filtrate (1).

It is noted in general that as tomatoes spoil the ammonia obtained by distillation with magnesium oxid increases in amount. The amount of ammonia so obtained is, for a pulp prepared from whole tomatoes, about twice as great as from a skin and core pulp. Thus, for pulped whole tomatoes this figure is found to be about 0.04 per cent, while for pulped skins and cores it runs about 0.02 per cent. Some other results which were obtained in attempting to follow the changes in the nitrogen bodies of tomatoes during spoilage are as follows:

(1) A solution of potassium ferro-cyanid and acetic acid gives no precipitate in the filtrates from the spoiled tomatoes FS–121–124 or with the filtrates of good ketchups. Boiling with a small amount of nitric acid gives no precipitate in the filtrate from either good or poor ketchups; the same is true of picric acid, phosphotungstic acid, and tannic acid and salt. There is evidently no protein in such filtrates.

(2) Nitrogen partition with basic lead acetate solution was determined as follows: Make up 25 grams of ketchup to 200 cc with water. Add 10 cc of 25 per cent solution of basic lead acetate. Filter through a 11 cm folded filter, wash four times, filling the filter each time with water, and determine total nitrogen in the filtrate and the precipitate.

Nitrogen partition with basic lead acetate.

Laboratory No.	Quality.	Nitrogen in filtrate.	Nitrogen in precipitate.	Ratio.
984–C	Poor	0.0376	0.0216	1.7 : 1
899–C	do	.0351	.0269	1.3 : 1
734–C	do	.0227	.0219	1.0 : 1
1694–C	do	.0365	.0269	1.3 : 1
8072–C	do	.0418	.0263	1.5 : 1
10325–C	Good	.0707	.0294	2.4 : 1
2540–C	do	.0671	.0278	2.4 : 1
9329–C	do	.0463	.0230	2.0 : 1
9330–C	do	.0536	.0202	2.6 : 1
21700–F	do	.0317	.0154	2.0 : 1

In the following table are found the results on some commercial products, for which only a few of the more significant determinations were made.

Significant determinations made on commercial products.

Substance and laboratory number.	Lactic acid.	Citric acid.	Volatile acids.	Reducing sugars.	Ammonia by the magnesium oxid method.	Remarks.[1]
Ketchup:	*Per ct.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
I. S. No. 2473–C	0.44					Molds, 92 per cent fields; yeast and spores, 250 per $\frac{1}{20}$ mm; bacteria, 25,000,000 per cubic centimeter.
I. S. No. 1342–C	.97					Molds, 60 per cent fields; yeast and spores, 74 per $\frac{1}{20}$ mm; bacteria, 72,000,000 per cubic centimeter.
I. S. No. 899–C	.56					Molds, 100 per cent fields; yeast and spores, 48 per $\frac{1}{20}$ mm; bacteria, 200,000,000 per cubic centimeter.
I. S. No. 1835–C	.3					Molds, 12 per cent fields; yeast and spores, 100 per $\frac{1}{20}$ mm; bacteria, 16,000,000 per cubic centimeter.
I. S. No. 733–C	.66					Molds, 100 per cent fields; yeast and spores, 250 per $\frac{1}{20}$ mm; bacteria, 35,000,000 per cubic centimeter.
I. S. No. 1542–C	.9	Traces.				Molds, 60 per cent fields; yeast and spores, 64 per $\frac{1}{20}$ mm; bacteria, 72,000,000 per cubic centimeter.
Tomato pulp, I. S. No. 9964–C.	.75	Traces.	0.22	0.0		Molds, 66 per cent fields; yeast and spores, 64 per $\frac{1}{20}$ mm; bacteria, 140,000,000 per cubic centimeter.
Tomato paste, I. S. No. 2551–C.	.77	None.	.78	0.0	0.0289	Molds, 52 per cent fields; yeast and spores, 300 per $\frac{1}{20}$ mm; bacteria, 650,000,000 per cubic centimeter.
Tomato pulp, I. S. No. 9963–C.	.71	Traces.	.193	Traces.		Molds, 60 per cent fields; yeast and spores, 56 per $\frac{1}{20}$ mm; bacteria, 156,000,000 per cubic centimeter.
Tomato paste:						
I. S. No. 9551–C	.8	None.	.18	None.	.034	Molds, 60 per cent fields; yeast and spores, 500 per $\frac{1}{20}$ mm; bacteria, 700,000,000 per cubic centimeter.
I. S. No. 9552–C	.84	None.	.19	None.	.031	
I. S. No. 9553–C	.79	None.	.19	None.	.032	
I. S. No. 2556–C	.73	.19	.16	None.	.0377	Molds, 72 per cent fields; yeast and spores, 250 per $\frac{1}{20}$ mm; bacteria, 200,000,000 per cubic centimeter.
Ketchup, I. S. No. 8098–C.	.621	Traces.			.057	Molds, 85 per cent fields; yeast and spores, 65 per $\frac{1}{20}$ mm; bacteria, 100,000,000 per cubic centimeter.

[1] Microchemical examinations made by B. J. Howard.

Significant determinations made on commercial products—Continued.

Substance and laboratory number.	Lactic acid.	Citric acid.	Volatile acids.	Reducing sugars.	Ammonia by the magnesium oxid method.	Remarks.
Tomato pulp:	*Per ct.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
I. S. No. 2485–C	0.1	.....	None.	.....	.....	Molds, 88 per cent fields; yeast and spores, 180 per $\frac{1}{66}$ mm; bacteria, 10,000,000 per cubic centimeter.
I. S. No. 3328–C	.25	.....	0.15	1.38	.....	Molds, 60 per cent fields; yeast and spores, 325 per $\frac{1}{66}$ mm; bacteria, 25,000,000 per cubic centimeter.
Ketchup, I. S. No. 1694–C.	.24	.....	.....	.....	.....	Molds, 70 per cent fields; yeast and spores, 189 per $\frac{1}{66}$ mm; bacteria, few.
Tomato paste, I. S. No. 9926–C.	.42	.....	.16	None.	.....	Molds, small; yeast and spores, 700 per $\frac{1}{66}$ mm; bacteria, 125,000,000 per cubic centimeter.
Canned tomatoes:						
I. S. No. 3324–C	.09	.....	.05	2.7	.....	Molds, traces; yeast and spores, 4 per $\frac{1}{66}$ mm; bacteria, 6,000,000 per cubic centimeter.
I. S. No. 3323–C	.1	.....	.05	2.78	.....	Molds, traces; yeast and spores, 9 per $\frac{1}{66}$ mm; bacteria, 10,000,000 per cubic centimeter.
I. S. No. 3322–C	.11	.....	.042	2.78	.....	Molds, traces; yeast and spores, 9 per $\frac{1}{66}$ mm; bacteria, 10,000,000 per cubic centimeter.
Tomato pulp:						
I. S. No. 881–C	.8	None.	.....	.....	.....	Molds, 94 per cent fields; yeast and spores, 329 per $\frac{1}{66}$ mm; bacteria, 129,000,000 per cubic centimeter.
I. S. No. 2481–C	.2	.....	.124	.....	.....	Molds, 70 per cent fields; yeast and spores, 379 per $\frac{1}{66}$ mm; bacteria, 29,000,000 per cubic centimeter.
I. S. No. 12426–C	.61	0.38	.36	None.	.....	Molds, 58 per cent fields; yeast and spores, 42 per $\frac{1}{66}$ mm; bacteria, 124,000,000 per cubic centimeter.
Tomato ketchup, I. S. No. 2259–C.	.17	.....	.....	.....	.....	Molds, 40 per cent fields; yeast and spores, 52 per $\frac{1}{66}$ mm; bacteria, 15,000,000 per cubic centimeter.
Tomato pulp, I. S. No. 2393–C.	.204	.....	.08	.....	.....	Molds, 14 per cent fields; yeast and spores, 60 per $\frac{1}{66}$ mm; bacteria, 25,000,000 per cubic centimeter.
Ketchup, I. S. No. 1836–C.	.08	.....	.....	.....	.....	Molds, 50 per cent fields; yeast and spores, 30 per $\frac{1}{66}$ mm; bacteria, few.
Tomato pulp:						
I. S. No. 2394–C	.36	.....	.09	None.	.....	Molds, 16 per cent fields; yeast and spores, 25 per $\frac{1}{66}$ mm; bacteria, 40,000,000 per cubic centimeter.
I. S. No. 2497–C	.04	.....	Traces.	.....	.....	Molds, 60 per cent fields; yeast and spores, 140 per $\frac{1}{66}$ mm; bacteria, very few.
Ketchup, I. S. No. 2258–C.	.13	.....	.....	.....	.....	Molds, 50 per cent fields; yeast and spores, 56 per $\frac{1}{66}$ mm; bacteria, 12,000,000 per cubic centimeter.
Tomato pulp, I S. No. 1484–C.	.24	.....	.066	1.7	.....	Molds, 8 per cent fields; yeast and spores, 100 per $\frac{1}{66}$ mm; bacteria, 25,000,000 per cubic centimeter.
Ketchup:						
I. S. No. 1364–C	.48	.....	.....	.....	.....	Molds, 50 per cent fields; yeast and spores, 53 per $\frac{1}{66}$ mm; bacteria, 129,000,000 per cubic centimeter.
I. S. No. 984–C	.55	.....	.....	.....	.....	Molds, 40 per cent fields; yeast and spores, 16 per $\frac{1}{66}$ mm; bacteria, 70,000,000 per cubic centimeter.

Significant determinations made on commercial products—Continued.

Substance and laboratory number.	Lactic acid.	Citric acid.	Volatile acids.	Reducing sugars.	Ammonia by the magnesium oxid method.	Remarks.
Ketchup—Continued.	*Per ct.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
I. S. No. 7945–C.....	.12	.....	.....	.....	.....	Molds, 25 per cent fields; yeast and spores, 150 per $\frac{1}{6\,6}$ mm; bacteria, few.
Tomato pulp:						
I. S. No. 9158–C.....	.51	Traces.	.18	None.	.....	Molds, 33 per cent fields; yeast and spores, 160 per $\frac{1}{6\,6}$ mm; bacteria, 400,000,000 per cubic centimeter.
I. S. No. 10604–C....	.5	Traces.	.18	None.	.....	Molds, 60 per cent fields; yeast and spores, 200 per $\frac{1}{6\,6}$ mm; bacteria, 250,000,000 per cubic centimeter.
I. S. No. 11049–C....	.52	None.	.54	None.	.....	Molds, 80 per cent fields; yeast and spores, 300 per $\frac{1}{6\,6}$ mm; bacteria, 132,000,000 per cubic centimeter.
I. S. No. 2494–C.....	.72	.....	.3	.....	.....	Molds, 54 per cent fields; yeast and spores, 70 per $\frac{1}{6\,6}$ mm; bacteria, 130,000,000 per cubic centimeter.
I. S. No. 18315–C....	.8	None.	.1	.....	.....	Molds, 85 per cent fields; yeast and spores, 350 per $\frac{1}{6\,6}$ mm; bacteria, 200,000,000 per cubic centimeter.

CITRIC ACID DETERMINATIONS.

The method which is proposed for the determination of citric acid in ketchups is as follows:

Weigh 25 grams into a 250 cc beaker, make up to approximately 200 cc with 95 per cent alcohol, allow to stand with frequent stirring for four hours, filter through a folded filter and wash with 50 cc of 80 per cent alcohol. To the filtrate add sufficient water to dilute the alcohol to 50 or 60 per cent and then add 10 cc of 20 per cent barium acetate solution, stir well with a glass rod, and allow to stand over night. In the morning filter on a Gooch crucible, washing with 50 per cent alcohol, dry for from 3 to 4 hours in an oven at 100° C. and weigh. Weight of precipitate times 0.51 equals anhydrous citric acid.

This method approximates very closely the amount of citric acid present in such a product, as was proved by sending out to a number of chemists solutions containing about 20 per cent of sugar and known amounts of citric and lactic acids. The amounts of citric acid found in these solutions were very close to the actual amount present.

Unfortunately there is at present no convenient method for the determination of citric acid in solutions of the type of a ketchup which gives absolutely accurate results. The method as given has been very thoroughly tested and has been found to give results of sufficient accuracy for the purpose at hand. It may not be amiss to give a few details as to attempts which were made to find a better method for the determination of citric acid. Beau[1] gives a method for the determination of citric acid in milk, using the Denigès[2]

[1] Rev. gén. du lait, 1904, p. 386; Chem. Centrbl., 1904, 75 (2): 856.

[2] Ann. chim. phys., 1899, (7) 18: 413.

procedure for the qualitative detection of citric acid. To 50 cc of milk add 75 cc of water and 50 cc of Denigès reagent and make up to 200 cc. Heat to boiling, and after removing the heat add drop by drop 1 per cent of potassium permanganate solution until the yellow precipitate quickly settles and the fluid clears. Boil and then add hydrogen peroxid to remove the excess of permanganate. Filter and weigh. The weight of the precipitate times 0.271 equals citric acid. This procedure was tried a number of times, both exactly according to directions and with different modifications, but the results were exceedingly unsatisfactory. The formation of the acetone dicarbonic acid mercuric sulphate complex was not quantitative under any conditions which were tried.

It seemed theoretically possible to dehydrate citric acid to give acetone dicarbonic acid, which, either under the influence of gentle oxidizing agents or simply by hydrolysis, might break down into acetone and carbon dioxid, when the acetone could be distilled off and estimated very exactly by the Dénigès method. A large number of experiments were made to test these suggestions, but the results were unsatisfactory. While citric acid does break down in this manner to a very large extent when treated with strong sulphuric acid or with glacial phosphoric acid, or even with dilute sulphuric acid and potassium permanganate, no conditions have yet been found under which this reaction proceeds quantitatively. It was found that the procedure of Beau was theoretically impossible as some acetone dicarbonic acid would, in the presence of sulphuric acid and potassium permanganate, break down into acetone, which forms a quite different salt with mercuric sulphate from the one formed by acetone dicarbonic acid, on which Beau bases his calculations.

Determination of citric acid by the method of Spica[1] in which citric acid or a citrate is decomposed by sulphuric acid at 100° C. and the evolved carbon monoxid gave satisfactory results with the gas apparatus devised by the authors,[2] as is shown by the following data:

0.36 gram of citric acid gave 44.0 cc of carbon monoxid at 27° C. and 766 mm, equivalent to 39.1 cc at 760 mm and 0° C., which equals 0.367 gram of citric acid or a recovery of 101.9 per cent.

0.248 gram of citric acid gave 29 cc of carbon monoxid at 21° C. and 772 mm, equivalent to 0.250 gram of citric acid, a 100.8 per cent recovery.

LACTIC ACID DETERMINATIONS.

The method proposed for the determination of lactic acid in ketchup and similar products is as follows:

To 100 grams of ketchup add 10 cc of 20 per cent normal lead acetate, make up to 500 cc. shake well and centrifuge. To 400 cc of the clear portion add a moderate excess

[1] Chem. Ztg., 1910, 34: 1141.

[2] U. S. Dept. Agr., Bureau of Chemistry Cir. 80.

of sulphuric acid, filter, wash the precipitate with a small amount of water, and evaporate the filtrate on the steam bath to a convenient volume for extraction in a continuous liquid extractor. (In this laboratory this volume is 100 cc.) Extract for from 18 to 20 hours in a liquid extractor with washed ether. In case the quantity of lactic acid present is greater than 0.5 gram it is usually necessary to extract for a longer period. In any case it is best to reextract for from 8 to 10 hours to make sure that the extraction is complete. (Ether sufficiently pure for this purpose may be prepared by shaking out ordinary ether once with a sodium hydrate solution and then 10 times with small quantities of water.) The ether is just evaporated on the steam bath, and the residue at once taken up in water and filtered. This procedure is made to get rid of a small amount of coloring matter and substances other than lactic acid which may be extracted from ketchup by ether but which are insoluble in water. The filtrate is heated on the steam bath for some time to remove all traces of ether or alcohol. Approximately 3 grams of sodium hydrate are then added to the water solution and 50 cc of a 1.5 per cent solution of potassium permanganate are added from a pipette. This mixture is heated on a water bath at 100° C. for one-half hour. At the end of that time, or before, if the color is not a decided blue-black or purple, but is green or colorless above the layer of brown precipitate, more standard permanganate in measured portions is added until, after heating one-half hour on a boiling water bath, the color is a blue-black or purple. The oxidation is then complete. The hot solution is strongly acidified with dilute sulphuric acid, about 50 cc of 10 per cent sulphuric acid, and standard oxalic acid run in from a burette until the solution is decolorized. (In this laboratory a 5 per cent solution of oxalic acid is used for this purpose.) Any slight excess of oxalic acid is titrated back with the same standard permanganate solution. It should be understood that any standard permanganate and oxalic acid solution may be used within reasonable limits of strength. In alkaline solution the permanganate oxidizes the lactic acid quantitatively to oxalic acid according to the equation:

$$2C_3H_6O_3+10KMnO_4=2(COOH)_2+4H_2O+2CO_2+5MnO_2+5K_2MnO_4.$$

Then in acid solution, the oxalic acid is further oxidized by the permanganate to carbon dioxid and water according to the equation:

$$5(COOH)_2+2KMnO_4+3H_2SO_4=10CO_2+8H_2O+K_2SO_4+2MnSO_4.$$

Calculation: The total weight of permanganate used in the oxidation of the lactic acid is determined by subtracting the permanganate equivalent of the oxalic acid used from the total amount used. The weight of permanganate times 0.237 equals the weight of lactic acid.

That the substance under oxidation in the proposed method is actually lactic acid was proved in several spoiled ketchups and in spoiled tomatoes by preparing the zinc salt, which has a rather characteristic form. Zinc was determined in several of these salts and the percentage present found to agree with that calculated for zinc lactate.

A large number of experiments were made on the determination of lactic acid which seem to be important in this connection. Several experiments were carried out with solutions containing from 0.1 to 1 per cent lactic acid. In such solutions lead or barium acetates give no precipitate either in aqueous solutions or in 80 per cent alcohol. Stannous chlorid gives no precipitate in dilute lactic acid or sodium lactate solutions. The zinc lactate is not easily or completely precipitated from weak lactic acid solutions (0.1 to 0.2

per cent). Hence, it was considered necessary to separate the lactic acid by extraction with an immiscible solvent.

The following experiments were made under the varying conditions specified:

(1) Used a 20 cc lactic acid solution which titrated 8 cc tenth-normal sodium hydroxid. Saturated with salt and then added sulphuric acid; extracted 5 times, using 20 cc of ether each time. Extraction 50 per cent.

(2) Saturated 25 cc of 1 per cent lactic acid with salt and extracted as above; extraction 50 per cent. Similar solutions made strongly acid with sulphuric or phosphoric acids gave practically the same extraction.

(3) Used 25 cc of 1 per cent lactic acid as in No. 2, but no salt or acid; extraction 30 per cent. Using acetic ether in the same manner instead of ether gave an extraction of 70 per cent.

(4) Acidified 25 cc of lactic acid, 1.8 per cent, with sulphuric acid, made up to 100 cc and extracted for 16 hours in the continuous extractor for liquids. Recovery from 98 to 100 per cent.

(5) Added to 100 grams of good ketchup containing no lactic acid 0.44 gram of lactic acid and treated as in the method given on page 9. Recovery, 0.41 gram, 91 per cent.

A number of special experiments have shown that when the extraction with ether is properly carried out no sugars will be found in the extract, even when the solutions extracted contain a high percentage of sugar.

BEHAVIOR OF LACTIC ACID TOWARD OXIDIZING AGENTS.

The following experiments were made to determine the behavior of lactic acid under different conditions:

(1) Ten cubic centimeters of lactic-acid solution, containing 0.12 gram of lactic acid, 8 grams of potassium hydroxid, and 40 cc of potassium permanganate solution (8.4 cc = 0.5 gram of oxalic acid), were heated on the steam bath for 30 minutes, and sulphuric acid added to strong acid reaction. Fifteen cubic centimeters of 10 per cent oxalic acid were then added and titrated with the permanganate solution described on page 10, 2.2 cc being required. Found 0.119 gram instead of the 0.12 gram added.

(2) Ten cubic centimeters of lactic-acid solution, containing 0.09 gram of lactic acid, oxidized with alkaline permanganate as described, gave 0.127 gram of oxalic acid ($H_2C_2O_4 + 2H_2O$) instead of the calculated 0.126 gram.

(3) Ten cubic centimeters of lactic-acid solution, containing 0.221 gram of lactic acid with 8 grams of potassium hydroxid and 40 cc of potassium permanganate solution (8.3 cc = 0.5 gram of oxalic acid), was heated for 10 minutes on the steam bath, an excess of sulphuric

acid and 10 cc of 10 per cent oxalic acid added, and titrated with the permanganate solution before described, 7.2 cc being required. Found 0.2203 gram instead of the 0.221 gram added.

(4) Ten cubic centimeters of lactic-acid solution containing 0.12 gram of lactic acid was made just alkaline to phenolphthalein and heated on the steam bath for 10 minutes with 20 cc of potassium permanganate solution (8.4 cc = 0.5 gram of oxalic acid). Five grams of oxalic acid were added and titrated with the permanganate solution, 1.7 cc being required. Found 0.094 gram of lactic acid instead of the 0.12 gram added.

(5) To 0.12 gram of lactic acid in 10 cc of water were added 10 cc of strong sulphuric acid, and the hot solution was titrated with potassium permanganate until a pink color, persistent for 5 minutes, was obtained. This required 5.8 cc of potassium permanganate solution (8.4 cc = 0.5 gram oxalic acid). Calculated, 16.7 cc were required for complete oxidation of the lactic acid.

(6) Proceeded exactly as in experiment 5 except that the 0.12 gram of lactic acid was dissolved in 100 cc of water. Required 4 cc of potassium permanganate solution for titration.

(7) A 10 cc lactic-acid solution containing 0.281 gram of lactic acid was made strongly acid with concentrated sulphuric acid and then titrated; 73 cc of potassium permanganate solution (69 cc = 0.5 gram of oxalic acid, $H_2C_2O_4 + 2H_2O$) were required, and large amounts of acetaldehyde were evolved during this titration. The amount of permanganate required was 63 per cent of the theoretical necessary to oxidize the lactic acid completely to carbon dioxid and water. Calculation shows, however, that only one-fourth of this permanganate would be necessary to oxidize the formic acid if the reaction is

$$CH_3CHOHCOOH \rightarrow CH_3CHO + HCOOH + O \rightarrow CH_3CHO + CO_2 + H_2O;$$

in fact much more permanganate was used than was necessary to oxidize all the lactic acid to acetic acid and carbon dioxid, according to the reaction

$$CH_3CHOHCOOH + O_2 \rightarrow CH_3COOH + CO_2.$$

It is necessary to assume that under the conditions of this experiment the lactic acid breaks down at once into small groups and not alone into acetaldehyde and formic acid.

(8) Twenty-five cubic centimeters of 1 per cent lactic acid and 25 cc of 5 per cent sulphuric acid were boiled and 20 cc distilled. No acetaldehyde was found. To obtain the acetaldehyde it was necessary to add manganese dioxid or some other similar oxidizing agent. It is evident that the decomposition of lactic acid represented by the equation

$$CH_3CHOHCOOH \rightarrow CH_3CHO + HCOOH$$

does not take place as easily as is usually assumed.

(9) To 0.010 gram of lactic acid in 20 cc of water were added 0.5 gram of sodium hydrate and a slight excess of potassium permanganate, the solution being heated on the steam bath for 10 minutes; the excess of permanganate was decomposed with hydrogen peroxid, the manganese dioxid filtered off and the filtrate made faintly acid with acetic acid, the oxalic acid being precipitated as calcium oxalate by the addition of calcium acetate. A strong precipitate of calcium oxalate was obtained. By the same procedure and careful working with small volumes, it was possible to obtain this calcium oxalate precipitate with 1 mg of either lactic or malic acid. A control oxidation in which only acetic acid was used gave no reduction of permanganate and no calcium oxalate.

Experiments 1, 2, and 3 prove that in alkaline solution lactic acid is oxidized quantitatively to oxalic acid by potassium permanganate solutions.[1] Experiment 4 proves that it is necessary to have present more than enough alkali to form the salt of lactic acid[2] and experiments 5, 6, and 7 show that any method of determining lactic acid in acid solution, such as that of Jerusalem,[3] would demand a very rigid adherence to certain conditions to obtain comparable results, and hence is of doubtful value for routine work.

With reference to the detection of lactic acid the much used Uffelmann test[4] does not seem to be wholly reliable, as the same reaction is obtained with oxalic, tartaric, succinic, and citric acids. The method of detection proposed by Herzog,[5] which depends on the reaction

$$CH_3CHOHCOOAg + I_2 \rightarrow CHOHCOOH + CH_3CHO + CO_2 + 2AgI,$$

the acetaldehyde being detected by known reactions, was found to be more satisfactory for qualitative work than the Uffelmann test.

THE BEHAVIOR OF MALIC AND TARTARIC ACID TOWARD OXIDIZING AGENTS.

(1) Used 10 cc of 1 per cent malic acid, 3 grams of potassium hydroxid, and 120 cc of potassium permanganate solution (69 cc = 0.5 gram of malic acid); proceeded as under alkaline oxidation of lactic acid; found 96.4 per cent of the theoretical amount.

(2) Proceeded exactly as in No. 1 except that 8 grams of potassium hydroxid were used; found 99.6 per cent of the theoretical amount present.

(3) Used 10 cc of 1 per cent malic acid as in No. 1; found 0.1063 gram of malic acid instead of 0.100 gram.

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[1] Ulzer and Seidel, Monatshefte für Chemie, 1897, 18:138.

[2] Denis, Amer. Chem. J., 1907, 38:561.

[3] Biochem. Zts., 1908, 12:361.

[4] Futh and Lochermann, Chem. Centrbl., 1906, 77(1):1452; ibid, 77(2):1087; Pharm. Ztg., 1910, 55:120.

[5] Ann. Chem. (Liebig), 1907, 351:263.

(4) Used 10 cc of a 1 per cent malic acid solution, 3 grams of sodium hydroxid, and enough potassium permanganate to produce a blue-black color. Heated for 30 minutes on the steam bath, removed excess of permanganate with hydrogen peroxid, and filtered. Determined the oxalic acid as calcium oxalate and found 0.190 gram of oxalic acid ($H_2C_2O_4+2H_2O$) instead of the calculated amount, 0.188 gram.

(5) Used 20 cc of 1 per cent malic acid, just as in No. 3; found 0.3747 gram of oxalic acid in place of the calculated amount, 0.376.

(6) Used 25 grams of 1 per cent malic acid, exactly as in No. 3; found 0.475 gram of oxalic acid instead of 0.470 gram as calculated.

(7) Samples Nos. 1, 2, 3, 4, 5, 6, each containing 10 cc of 1 per cent malic acid, and, respectively, 5, 10, 15, 20, 25, and 30 cc of 10 per cent sodium hydroxid solution and 20 cc of permanganate solution (8.5 cc = 0.5 gram oxalic acid). Heated for fifteen minutes on a steam bath, acidified with sulphuric acid and added to each sample 10 cc of 10 per cent oxalic acid. To titrate back required for No. 1, 7.8 cc; No. 2, 7.7; No. 3, 7.7; No. 4, 7.8; No. 5, 7.7; and No. 6, 7.7 cc of this permanganate, respectively. Found 0.096 and 0.097 gram. The variation of the amount and strength of the alkali within the limits given has no effect on the course of the oxidation.

When, however, the amount of alkali present is just sufficient to neutralize the malic acid, the oxidation to malic acid does not take place quantitatively, as is shown by the following experiments:

(*a*) Used 10 cc of 1 per cent malic acid, just neutralized to phenolphthalein, with sodium hydrate; oxidized with permanganate exactly as in experiment 1. Found 0.058 gram instead of 0.100 gram.

(*b*) Used 10 cc of 1 per cent malic acid just as in (*a*). Found 0.078 gram instead of 0.100 gram.

The oxidation of malic acid in acid solution with potassium permanganate is very similar to that with lactic acid; the reaction does not yield carbon dioxid and water quantitatively as is shown by the following experiments:

(*a*) Used 10 cc of 1 per cent malic acid with 5 cc of concentrated sulphuric acid, requiring 66 cc of potassium permanganate solution (69 cc = 0.5 gram of oxalic acid) for oxidation to a pink color permanent for five minutes. Calculated for complete oxidation to carbon dioxid and water, 77.8 cc.

(*b*) Used 10 cc of 1 per cent malic acid and 10 cc of concentrated sulphuric acid same strength as in (*a*). Required 59 cc of potassium permanganate; calculated amount, 77.8 cc.

Tartaric acid exhibits the same general behavior as malic and lactic acids toward acid and alkaline permanganate solutions, as is shown by the following experiments:

(*a*) Used 10 cc of 1 per cent tartaric acid, 8 grams of sodium hydroxid, and 20 cc of potassium permanganate solution (8.4 cc = 0.5

gram of oxalic acid). Heated 15 minutes on a steam bath, added 1 gram of oxalic acid, and made strongly acid with sulphuric acid. To titrate back required 4 cc of the potassium permanganate. Found 0.1003 gram instead of 0.100 gram.

(*b*) Used 0.293 gram of tartaric acid in 100 cc of water, 3 grams of sodium hydroxid, and enough potassium permanganate to produce a blue-black color (about 1.8 gram). Heated 30 minutes on the steam bath, added hydrogen peroxid to destroy excess of potassium permanganate, boiled to remove excess of hydrogen peroxid, filtered, made the filtrate acid, and determined oxalic acid by titration with potassium permanganate. Found 0.485 gram of oxalic acid instead of the calculated amount, 0.492 gram; 98.5 per cent yield.

(*c*) Used 10 cc of 1 per cent tartaric acid and 10 cc of concentrated sulphuric acid. Potassium permanganate solution (8.4 cc = 0.5 gram of oxalic acid) was run into the hot solution to a permanent pink color. Required 5.3 cc; calculated for complete oxidation, 6.3 cc.

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